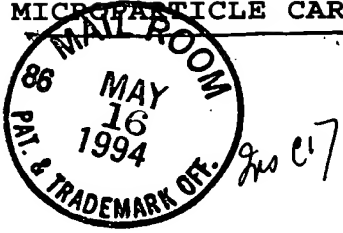


MICROPARTICLE CARRIERS OF MAXIMAL UPTAKE CAPACITY BY BOTH
M CELLS AND NON-M CELLS



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I. GOVERNMENT INTEREST

The invention described herein may be manufactured, licensed and used by or for governmental purposes without the payment of any royalties to us thereon.

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II. CROSS REFERENCE

This application is a continuation-in-part of U.S.

Patent Application Serial No. 07/867,301 filed April 10, 1992,
new U.S. patent 5,417,986,
which in turn is a continuation-in-part of U.S. Patent
filed November 21, 1986, now-abandoned,
Application Serial No. 07/805,721, which in turn is a
continuation-in-part of U.S. Patent Application Serial No.
07/690,485 filed April *24*, 1991, *now-abandoned,* which in turn is a
continuation-in-part of U.S. Patent Application Serial No.
07/521,945 filed May 11, 1990, *now-abandoned,* which in turn is a
continuation-in-part of U.S. Patent Application Serial No.
07/493,597 filed March 15, 1990, *now-abandoned,* which in turn is a
continuation-in-part of U.S. Patent Application Serial No.
06/590,308 filed March 16, 1984, *pending.*

III. FIELD OF THE INVENTION

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The invention pertains in part to a method for preparing particle size distributions of microparticles of biodegradable polymers having the capacity to be maximally absorbed in both M cells and non-M cells in the Peyer's patches (PP) follicle-associated epithelium (FAE) and the villous epithelium region so that when the microparticles are used as carries of

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immunogens for oral immunization, the maximal conditions for uptake by gut lymphoid tissues will absorb any antigens so as to induce production of antibodies against diseases caused by the antigen or other enteropathogenic organisms, when using
5 antigens encapsulated within biodegradable-biocompatible microspheres prepared by the process of the invention.

IV. BACKGROUND OF THE INVENTION

Infectious agents generally have their first contact with
10 host organisms at a mucosal surface. Therefore, mucosal protective immune mechanisms are of key importance in preventing these agents from colonizing or penetrating the mucosal surface. It is apparent from past studies that a protective mucosal immune response can best be obtained by
15 introduction of the antigen at the mucosal surface; however, parenteral immunization has not been an effective method to induce mucosal immunity. Antigen taken up by the gut-associated lymphoid tissue (GALT), primarily by the Peyer's patches stimulates T helper cells (T_H) to assist in IgA B cell
20 responses or stimulates T suppressor cells (T_{KS}) to mediate the unresponsiveness of oral tolerance.

While particulate antigen appears to shift the responses towards the (T_H), soluble antigens favor a response by the (T_{KS}).

25 Although studies have demonstrated that oral immunization does induce an intestinal mucosal immune response, large doses of antigen are generally required to achieve sufficient local concentrations in the Peyer's patches. Further, unprotected

protein antigens tend to be degraded or they complex with secretory IgA in the intestinal lumen.

One approach to overcoming the aforementioned problems is to homogeneously disperse the antigen of interest within the polymeric matrix of biodegradable, biocompatible microspheres that are specifically taken up by GALT. Eldridge, et al.¹ have used a murine model to show that orally-administered 1-10 micrometer microspheres consisting of polymerized lactide and glycolide, (the same materials used in resorbable sutures), were readily taken up into Peyer's patches, and that 1-5 micrometer sizes were rapidly phagocytized by macrophages. Microspheres that were 5-10 micrometers (microns) remained in the Peyer's patches for up to 35 days, whereas those less than 5 micrometer disseminated to the mesenteric lymph node (MLN) and spleen within migrating MAC-1⁺ cells.

However, Eldridge, et al. used 50 μ m microspheres of poly (DL-lactide-co-glycolide) composed of molar parts of polymerized lactide and glycolide (85:15 DL-PLG), which biodegrades to completion in approximately 24 weeks after intramuscular injection.

Poly (DL-lactide-co-glycolide) composed of equal molar parts of polymerized lactide and glycolide (50:50 DL-PLG) is the more stable or least biodegradable, and biodegrades to completion after 25 weeks.

Therefore, there is a need extant in the biodegradable microsphere field to provide a method of producing poly (DL-

¹ Biodegradable Microspheres: Vaccine Delivery System For Oral Immunization, 1989, 146.

lactide-co-glycolide) materials of 50:50 DL-PLG that is more biodegradable and capable of being taken up by both M cells and non-M cells in the Peyer's patches follicle-associated epithelium when used as microencapsulant as carriers for antigens for enteric immunization.

V. SUMMARY OF THE INVENTION

One object of the invention is to provide a method for producing microparticles of biodegradable-biocompatible microspheres having an average particle size distribution that maximizes uptake of the microspheres by both M cells and non-M cells, either in the villous epithelium or in the Peyer's patches follicle-associated epithelium so that, upon encapsulating antigens or other chemotherapeutic agents within these microspheres, large doses of antigen will not be required to achieve sufficient local concentrations in these regions of the intestines when these microparticles are used as carriers of immunogens for oral or other types of immunization.

A further object of the invention is to provide a method for producing microspheres composed of poly (DL-lactide-co-glycolide) having an average particle size distribution so as to maximize the uptake of these microspheres into the lymphoid tissue of the gut through uptake by both M cells and non-M cells, either in the villous epithelium or in the PP follicle-associated epithelium, in order to enable smaller doses of antigen to achieve sufficient local concentrations in these regions of the intestines when using the poly (DL-lactide-co-

glycolide) as a carrier of immunogens for oral or other types of immunization.

A yet further object of the invention is to provide a method for producing an average distribution of particle sizes of the most stable or least biodegradable poly (DL-lactide-co-glycolide) having equal molar parts of polymerized lactide and glycolide (50:50 DL-PLG) so as to maximize uptake of microspheres of this copolymer by both M cells and non-M cells, either in the villous epithelium or in the PP follicle-associated epithelium when using this copolymer as a carrier of immunogens for oral or other types of immunization in mammals.

In general the invention is accomplished by modifying the solvent extraction process for producing microspheres so that the average particle size distribution can be controlled by altering the viscosity of the emulsion, either by: 1) pre-dilution of the emulsion oil with extractant solvent; 2) adding thickening agents such as polybutylene to the emulsion oil to deliberately increase its viscosity; 3) use of oils with predefined viscosities for preparation of the emulsion; or 4) by deliberately adjusting the viscosity of paraffin oil used by preheating it to a temperature which yields the desired viscosity. When the emulsion time is kept sufficiently short to prevent a significant temperature increase during the emulsification process, the oil viscosity is the primary process parameter in determining the average distribution of particle size ranges of the spheres' diameter. Variations in screen and rotor dimensions of the equipment and

emulsification speed and time have negligible effects on the outcome of the microspheres diameter.

VI. BRIEF DESCRIPTION OF THE DRAWINGS

5 Fig. 1 shows that, during preparation of the microspheres, the spheres actually got larger as the emulsion time was increased.

 Fig. 2 is a schematic showing the preparation of sucrose-loaded vaccine placebo microspheres.

10 Fig. 3 is a graph showing substitution of stable-viscosity machine oils or paraffin oils during the formation of the emulsion to accomplish sphere populations whose average sizes and volumes decreased with increasing emulsification times, in contrast to that which was observed for emulsions
15 formed with paraffin oil as shown in Fig. 1.

 Fig. 4 shows the consistent relationship between sphere sizes at 3.0 minutes versus 0.75 minutes across all viscosities of oil tested, and show that sphere sizes are directly related to viscosity.

20 Fig. 5 shows that reducing the viscosity of the paraffin oil by diluting it with heptane resulted in the formation of progressively larger spheres.

 Fig. 6 shows that reducing the viscosity of the paraffin oil by diluting it with iso-octane resulted in the formation
25 of progressively larger spheres.

 Fig. 7 shows that when reducing the viscosity of the paraffin oil by diluting it with heptane using one second emulsification without an emulsion screen, resulted in the

formation of progressively larger spheres.

Fig. 8 shows microsphere volume average versus emulsification time in paraffin oil.

Fig. 9 shows viscosity versus sphere diameter obtained with paraffin oil diluted with iso-octane.

Fig. 10 shows viscosity versus sphere diameter obtained with paraffin oil diluted with heptane.

Fig. 11 shows viscosity versus sphere diameter obtained with machine oils.

Fig. 12 is a color photograph of the flank region of the intestinal lymphoid follicle of a New Zealand white rabbit histochemically stained for acid phosphatase (red) and immunohistochemically stained for the MHCII antigen.

Fig. 13 is a color photograph of the flank region of the intestinal lymphoid follicle histochemically stained for alkaline phosphatase (red) and immunohistochemically stained for the MHCII antigen.

Fig. 14 is a color photograph of the flank region of the intestinal lymphoid follicle of a New Zealand white rabbit showing numerous microspheres of the poly (DL-lactide-co-glycolide) composed of molar parts of polymerized lactide and glycolide (50:50 DL-PLG) in the company of MHCII-positive cells in lymphoid pockets in the Follicle Associated Epithelium (FAE), and wherein some of the microsphere particles are within the cells (arrows). In the lymphoid follicle, numerous MHCII-positive cells are present, and some have microspheres associated with them (arrowheads).

Fig. 15 is a color photograph showing that both kinds of

particles were taken up by the follicle-associated epithelium and entered the underlying lymphoid tissues of Peyer's patches (fluoresceinated microspheres are more easily visualized, and as a consequence they are shown in the photograph).

5 Fig. 16 is a color photograph showing the flank region of the previously illustrated intestinal lymphoid follicle and the adjacent villous stained for acid phosphotates (red) and CD43 (pan-T cell). Numerous CD43-positive cells are present in the FAE and in the lymphoid follicle. Microparticles in
10 the FAE are in the company of CD43-positive cells in the lymphoid pockets, and some of the particles are within the cells (arrows). The CD43 cells are CD8- and CD4-negative, and Igu-positive cells are sparse in the FAE. The microparticles have not entered the epithelium of the villous (v) adjacent to
15 the lymphoid follicle, although some are present nearby in the lumen.

 Fig. 17 is an immunofluorescence micrograph of the previously illustrated lymphoid follicle. The fluorescein-labeled microspheres are present mostly in the flank region of
20 the FAE (lower area of the photograph), with declining numbers present in the more apically located regions.

 Fig. 18 is a color photograph showing the lymphoid follicle of a Peyer's patch of a New Zealand white rabbit stained for vimentin. The polymerized lactide and glycolide
25 particles appear principally in the FAE area and are practically non-existent in the villous area.

 Fig. 19 is a color photograph of the lymphoid follicles of the New Zealand white rabbit's intestines showing the pan-T

cell markup stained for CD43. The view shows the villous epithelium, the lamina propria, the location of the copolymer (PLGA) particles and the CD43-positive cells.

VII. DETAILED DESCRIPTION OF THE INVENTION

Use of the emulsion viscosity as the means for controlling the average particle size distribution of polymerized lactide and glycolide microspheres has utility in manufacturing oral and injectable vaccines as well as for use in devices for sustained drug and antibiotic delivery. Preparation of the microspheres was accomplished by a modification of the solvent extraction process to control the sphere size by altering the viscosity of the emulsion either by: 1) pre-dilution of the emulsion oil with an extractant solvent; 2) adding thickening agents such as polybutylene to the emulsion oil to deliberately increase its viscosity; 3) through use of oils with predefined viscosities for preparation of the emulsion; or 4) through deliberately adjusting the viscosity of the paraffin oil by preheating it to a temperature which yields the desired viscosity, taking care that the emulsion time is kept sufficiently short so as to prevent a significant temperature increase during the emulsification process.

It has been found that the oil viscosity is the primary process parameter for controlling the sphere diameter, and that variation in screen and rotor dimensions, emulsification speed and time only exhibit negligible effects on the outcome of the diameter of the microspheres.

The following examples will provide more detailed steps in producing the controlled particle size microspheres of poly (DL-lactide-co-glycolide) by the modified solvent extraction process of the present invention.

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EXAMPLE:

SOLVENT EXTRACTION

PREPARATION OF FREEZE-DRIED ANTIGEN-SUCROSE MATRIX

Materials: 8 ml water
 80 mg sucrose
 20 mg purified antigen/active

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The freeze-drier is turned on and the temperature is set at -25 degrees.

PREPARATION OF THE ANTIGEN-SUCROSE MATRIX

15 The antigen/active is placed in a 20 ml capacity plastic vial to which water and sucrose are added.

The dispersion is then flash freezed by gently swirling the vial (without the cap) in liquid nitrogen for about one half of an hour.

20 After about 1000 minutes the temperature is elevated to about +5 degrees for 500 minutes (8.33 hours) and then elevated to about +20 degrees for 1000 minutes (16.67 hours), and the vial is removed.

25 **Preparation of Polymerized lactide glycolide (PLG) solution**

The PLG is removed from the freezer and allowed to come to room temperature.

About 2.8 g of acetonitrile is weighed into a 20 ml

capacity glass vial and set aside.

After the polymer reaches room temperature, about 1.0 g of the polymer is added to the vial of acetonitrile and a sonicator bath until all of the polymer has dissolved (5-10 minutes).

HOMOGENIZATION OF SUCROSE

Preparing the homogenizer

HOMOGENIZATION

3.2 g of acetonitrile is weighed in a plastic vial for washings during homogenization.

1.5 g of acetonitrile is weighed into another vial and added to the earlier prepared freeze-dried sucrose-antigen matrix and mixed until it becomes a milky white slurry. The slurry is homogenized at maximum speed for one minute and the 3.2 g of acetonitrile is used to wash the sides of the vial and homogenizer tip, after which the slurry is again homogenized for one minute at maximum speed.

The mixture is separated into two parts by weight by weighing 2.4 g into another 20 ml plastic vial.

The polymer solution prepared earlier is added to one of the vials of homogenized sucrose-antigen and the vial is placed in a sonicator bath for about 2 minutes to ensure proper mixing.

PREPARATION OF MICROSPHERES

The homogenizer is set up with the rotor and fine emulsion screen and the following materials are weighed out:

400 g of light mineral oil in a 600 ml glass beaker and 2500 g of heptane in a 4000 ml propylene beaker. A beaker of heptane is placed under the mixer and a propeller is placed about two-thirds of the way down into the heptane, after which the mixer is started at about 450 rpm.

A Masterflex mode #7550-60 peristaltic pump with pump head model #7518-00 with PharMed tubing, size 16 is set up. The pump speed is set at 300 ml/min after which one end of the tubing is placed into the beaker of heptane.

Approximately 175 ml of the mineral oil is poured into the jacket beaker and the homogenizer head is dipped at 15 degrees into the oil to coat it, after which water is circulated through the beaker.

The polymer/sucrose-antigen solution is poured into the beaker and the vial is rinsed with about 5 ml of mineral oil, and the rinse is added to the beaker.

The homogenizer head is placed into the liquid and then turned to its maximum setting for 3 minutes.

At the end of three minutes the other end of the tubing is placed into the jacketed beaker and the peristaltic pump is started. When the liquid level has dropped to the level of the homogenizer head, the homogenizer is turned off and pumping is continued until all of the liquid has been pumped to the heptane after which the heptane is left stirring for 30 minutes.

Using fresh tubing, the heptane is pumped into centrifuge bottles and centrifuged for 5 minutes at 3000 rpm, 20 degrees celsius. The supernatant is pumped into waste bottles and the

sediment is rinsed with heptane (it may be necessary to sonicate the sample for 1 to 5 minutes to break up the sediment).

5 The supernatant is pumped into the waste bottle and washed with fresh heptane until all the microspheres are in the one tared 50 ml centrifuge tube. This tube is then centrifuged for 5 minutes and washed with fresh heptane three times.

10 After the final wash and centrifuge cycle, the supernatant is pumped into the waste bottle and the microspheres are air dried with a slow air current for about 5 minutes, and the tube is placed in the vacuum oven at room temperature and left overnight.

15 The microspheres are removed from the vacuum oven and weighed, after which about 1 mg of the microspheres is put in a 1.5 ml centrifuge tube for evaluation.

EVALUATION OF MICROSPHERES

20 About 1 ml of 1% Tween 80 in water is added to the 1 mg of microspheres in the 1.5 ml centrifuge tube, and the tube is sonicated for about 1 minute.

One drop of the dispersion is placed on a glass slide and a coverslip is placed over it. The slide is then placed under a calibrated optical microscope and examined under 100X
25 magnification using a standard oil immersion technique. Using the precalibrated eyepiece micrometer, the diameter of 150 randomly chosen microspheres is determined. (Under 100X magnification, 1 division on the micrometer is equal to 1

micron.)

The numbers are then entered into a Lotus spread sheet program to determine the average size distribution of the particles.

5 The prior art extraction procedure for production of poly-lactide: glycolide microencapsulated oral vaccines is based on disbursal of a highly concentrated solution of polymer and acetonitrile into oil, followed by extraction of the acetonitrile and oil with heptane.

10 The procedure of the invention requires high energy shear to disburse the viscose polymer solution. This high shear process resulted in the generation of major heat change which caused the mineral oil's viscosity to change significantly.

15 As a result, small increases in shear time or minor differences in the emulsifier's rotor dimensions which increased shear, resulted in increased microsphere diameters, as can be seen from the graph of Fig. 16.

20 The substitution of stable viscosity machine oils for paraffin oil during the formation of the emulsion resulted in sphere populations whose average sizes and volumes decreased with increasing emulsification times.

25 This result can be seen in Table I, which is in contrast to the data showing microsphere volume average versus emulsification time and paraffin oil (Fig. 16).

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Table 1

<u>C/S*</u>	<u>Emulsification Time</u>			
	<u>0.75 minutes</u>		<u>3.0 minutes</u>	
	<u>V.A.**</u>	<u>D.A.***</u>	<u>V.A.</u>	<u>D.A.</u>
36	2.8	0.9	2.4	0.9
50	7.3	2.9	6.8	3.2
65	7.9	3.3	6.9	2.9
72	4.9	1.0	3.0	0.9
80	2.4	1.1	1.4	0.9

* = Centistokes

** = Volume Average

*** = Diameter Average

Both paraffin emulsions and machine oil emulsions underwent similar temperature increases during the emulsion process, and the differences between these two oils appears to be due to maintenance of a relatively constant emulsion viscosity by the machine oils. At a constant viscosity, increased homogenization time appears to have resulted in a progressively finer dispersal of the polymer-acetonitrile solution into the oil. Viscosity breakdown in the paraffin oil appears to have allowed particles to recombine as the emulsion temperature increased.

Reducing the viscosity of the paraffin oil by diluting it with either heptane or iso-octane resulted in the formation of a progressively larger spheres as can be seen in Table 2.

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1170x

Table 2

Sphere Diameters Resulting From Dilution of the Emulsion Oil

	Solvent/ Oil Mixture	Diameter Averages in U		
		H*	10**	10***
5	1/2	11.0	4.6	9.7
	1/4	2.6	5.0	3.3
	1/8	1.5	2.2	2.6
	1/16	1.2	1.4	0.6
	No Solvent	1.0	1.0	0.6

* = Heptane Diluent, ** = Iso-octane,
*** = 2nd Series of Iso-octane Batches Employing
Reduced Shear Forces

The results of these tables show that sphere size can be controlled by altering the viscosity of the emulsion oil through its pre-dilution with an extractant solvent, provided that the emulsion time is kept sufficiently short so as to prevent a significant temperature increase during the emulsification process.

The data in Table 1 shows a relationship between the microsphere size and oil viscosity in that, microsphere size increased as oil viscosity increased from 36 to 65 centistokes and then decreased from 65 to 80 centistokes, which appears to indicate a bell-shaped sphere size distribution as viscosity increased.

Fig. 17 shows viscosity versus sphere diameter obtained with paraffin oil diluted with iso-octane.

Fig. 18 shows viscosity versus sphere diameter obtained with paraffin oil diluted with heptane.

Fig. 19 shows viscosity versus sphere diameter obtained with machine oils.

A histochemical and immunohistochemical analysis of the uptake of PLG and polystyrene microparticles by Peyer's

patches from a New Zealand white rabbit was conducted using the poly (DL-lactide-co-glycolide) copolymer in which the molar parts of polymerized lactide and glycolide were 50:50, as prepared according to the modified solvent extraction process of the invention.

Fluorescent polystyrene microspheres were also used as a comparison to test these microparticles as carriers of immunogens for oral immunization, and to ascertain or determine the actual location of their uptake by gut lymphoid tissues, and to ascertain which tissues were engaged in the uptake.

The study also served in part to ascertain if encapsulation may protect the antigens from proteolytic degradation in the gut lumen and facilitate their uptake and retention in the intestinal lymphoid tissues, as a thorough understanding of the fate of ingested antigen-containing microparticles is important in using antigens which have been microencapsulated for enteric immunization strategies.

VIII. METHOD

Fluorescent polystyrene microspheres and unlabelled poly (lactide-co-glycolide) microspheres of diameters of 0.5, 1, and 2um were instilled into the lumens of in situ rabbit intestinal loops.

After a period of between about 1 to 2 hours, the loops were removed, and sections were cut and reacted histochemically for acid (AcP), phosphatase and immunohistochemically in a biotin-streptavidin method with

several monoclonal antibodies to the rabbit lymphoid cell antigens.

The rabbits were anesthetized New Zealand white rabbits and the dimensions of the intestinal loops were 2cm (containing Peyer's patches) and the tissue blocks were excised and fixed in periodate-lysine-paraformaldehyde.

The results of these tests show that both kinds of particles ($0.5 > 1 > 2 \mu\text{m}$) were taken up by the Peyer's patches.

However, the particles of copolymer from the invention process principally taken up in the Peyer's patch region have a volume average of about 1.0 to about 7.0 micrometers as the particle size distribution.

Particles of copolymer from the invention process principally taken up in the villous epithelium of the intestines have a volume average of from about 0.5 to about 2.0 micrometers.

Tables 3 and 4 show respectively, the particles used when testing placement in Peyer's patch and villous regions.

TABLE 3

Microspheres: Particle Size Distribution by Microscopy

Formulation: PL13000 1.0um

Date of Mfg: 13 DEC

Date Analysis: 13 DEC

Analyst: M.J. Van Houten

STAGE Magnification: 100 X

Calibration: 1 div = (u)

Batch: 00321 116

File: PL13000.0

1.00 11/16

between or equal to 5-10 u

%volume %number

73.25 13.33

greater than 10 u

%volume %number

0.00 0.00

between or equal to 1-5 u

%volume %number

26.75 86.67

Reading Frequency Bias u dn3 EDC3 %Vol. Dist. .01% fed F+D % Num Dist.

1	40	1	40	0	0	40	27
2	41	2	329	3	0	82	27
3	25	3	675	6	2	75	17
4	13	4	832	7	4	52	9
5	11	5	125	11	14	55	7
6	9	6	216	16	35	54	6
7	5	7	343	14	48	35	3
8	1	8	512	4	22	8	1
9	1	9	729	6	44	9	1
10	4	10	1000	33	329	40	3

Total	150	55	3025	12150	85	498	450	100
average		5.30	6.71		2.15		3	2.15
S.D.		2.87	6.88		2.08		2.85	2.12

Particle Size Distribution

AGGREGATION DATA

Vol % Aggregated

0.00

Height X

Volume Average (u)

7.9

Number Average (u)

3.0

Number % Aggregated

0.00

number X

Avg Part Size Aggregated

ERR

Avg # of Part per Aggregate

ERR

The significance of what particle size distribution of the copolymer prepared according to the invention process is taken up in the villous epithelium section of the intestine is that, for oral administration of a vaccine (especially when no booster vaccine is administered), the antigen must principally be uptaken by the villous epithelium region, which is more than 90% of the area of the intestine needed for effective immunization. On the other hand, the fact that some of the smaller particle size distribution copolymer materials are also taken up by the Peyer's patch region of the intestine while the majority of the copolymer is taken up by the villous epithelium section indicates that several combinations of modes of immunization may be effected through vaccine.

The following information obtains from a immunohistochemistry basis:

IMMUNOHISTOCHEMISTRY

Antibodies:

<u>Monoclonal Antibody</u>	<u>Source</u>	<u>Antigen Recognized</u>
V9	Biomed	Vimentin (M cell marker)
L11-35	Serotec	CD43 (pan T cell)
45-3	Spring Valley	MHC11
Ken-4	Spring Valley	CD4
12C7	Spring Valley	CD8
NRBM	Serotec	Ig u chain

Procedure:
Biotin-streptavidin method

Uptake was greatest along the flanks of the follicles, where M cells (demonstrated by anti-vimentin MAb) were most numerous. While the particles were sometimes present within M cell cytoplasm, they were much more numerous in the lymphocyte pockets of the M cells.

In the pockets, the particles were intermingled with cells that were CD43+, CD8-, CD4-, Igu-, and MHC II+.

The results showed that, occasionally, the particles were present within large AcP+ cells in the pockets. In the
5 follicular tissue beneath the M cell-rich epithelium, particles were very numerous in the vicinity of MHC II+ cells and occasionally within the large AcP+ cells.

Unexpectedly, microspheres also entered non-M cell epithelium cells, especially in the domes. These cells were
10 vimentin negative AcP+. Microparticles were sparse or absent in the subepithelial tissue beneath the cells.

As a result of these tests, it became clear that both M cells and non-M cells in the rabbit PP follicle-associated epithelium can take up certain microparticles. Only the M
15 cells may be capable of permitting migration of the particles to adjacent cells.

Microparticles taken up by the M cells appear to migrate to lymphocyte pockets richly populated with MHC II+ cells and CD8-/CD4- T cells, as well as to a certain extent to AcP+
20 phagocytic cells.